Mini-Review

Serum Marker Panels for Predicting Liver Fibrosis – An Update

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Abstract
Fibrosis prediction is an essential part of the assessment and management of patients with chronic liver disease. Traditionally the gold standard for assessment of fibrosis is liver biopsy, but it suffers from various limitations including risk of patient injury and sampling error. As a result, noninvasive tests of hepatic fibrosis have been used in patients with chronic liver disease due to conditions such as hepatitis B and C, and alcoholic and non-alcoholic fatty liver disease. With the advent of new direct-acting antivirals, hepatic fibrosis staging is an important component of treatment decisions in the care of patients with chronic hepatitis C virus infection. Current limitations of the noninvasive biomarker models include a significant indeterminate range, and a predictive ability that is limited to only a few stages of fibrosis. However newer technologies and novel proteins identified by proteomics and genomics offer the possibility for further refinement and individualisation of biomarker fibrosis models in the future.

Introduction

All patients with a chronic liver disease such as hepatitis B or C or non-alcoholic fatty liver disease (NAFLD) are at risk of developing advanced liver fibrosis or cirrhosis. The severity of hepatic fibrosis is known to affect long-term outcomes and hence clinical management and treatment. The complications of advanced fibrosis such as portal hypertension, ascites and hepatocellular carcinoma are all associated with reduced survival.[1] Therefore, clinical decision-making is to a large extent based on accurate staging of liver fibrosis. Over recent years, noninvasive biomarkers for the diagnosis of liver fibrosis have been developed, initially in chronic viral hepatitis. These have since seen their use expanded to include all causes of chronic liver disease. Most of the current panels of liver fibrosis markers are accurate only in detecting advanced fibrosis to cirrhosis stages.

Chronic Hepatitis C

Chronic hepatitis C is a blood-borne disease; most patients in Australia are infected with hepatitis C virus (HCV) through intravenous drug use. It is estimated that there were 227,000 patients with chronic hepatitis C in Australia in 2019.[2] The burden of liver disease due to HCV infection is projected to triple by 2030.[3] HCV infection causes long-term liver damage and may result in progression from liver fibrosis to cirrhosis. Approximately 5–30% of patients with chronic hepatitis C develop advanced fibrosis or cirrhosis over 20–30 y after initial infection. It is estimated that HCV infection results in 2550 deaths per year in Australia.[4]

Chronic Hepatitis B

Chronic hepatitis B is also a blood-borne disease and the common transmission routes include vertical transmission at birth, sexual transmission and intravenous drug use. It is estimated that 230,000 individuals had chronic hepatitis B in Australia in 2016 with 15–20% of patients developing cirrhosis during their lifetime.[1] The primary goal of treating hepatitis B patients is to improve patient survival by preventing or delaying the development of cirrhosis and liver cancer.

New Treatments for Chronic Hepatitis

Recent Australian guidelines recommend that direct-acting antivirals (DAA) anti-HCV treatment should be offered to all patients with chronic hepatitis C infection. It is also recommended that noninvasive methods are used to determine the presence of cirrhosis in patients with chronic hepatitis C prior to commencing DAA treatment.[5]

The availability of DAA medicines has had important implications for the treatment of hepatitis C. DAA therapy

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has a high efficacy in achieving sustained virologic response (SVR) with improved safety compared to earlier therapy options. The new generation of DAA therapy is reported to achieve high real-world SVR rates (>90%) at 12 w across all genotypes and in individuals with cirrhosis and patients who had previously been treated.[6] All patients with chronic HCV infection are eligible for treatment, regardless of liver fibrosis stage, although the presence of cirrhosis influences treatment duration and regimen. A patients's cirrhosis status must be provided at the time of seeking Pharmaceutical Benefits Scheme (PBS) authority to write a prescription for the new HCV medicines.

The presence or absence of cirrhosis has been incorporated into the PBS authority requirements for the treatment of hepatitis B; this information must be provided for all patients at the time of application for PBS treatment with tenofovir, entecavir, lamivudine and adefovir. There are two groups of drugs used to treat hepatitis B: direct antiviral drugs (nucleoside/nucleotide analogues) and immunomodulatory drugs (interferons). Nucleoside/nucleotide analogues reduce the amount of hepatitis B virus in the body by lowering the ability of the virus to multiply. Interferons are proteins that modify the response of the body's immune system to help fight infections and severe diseases. In Australia, pegylated interferon has replaced standard interferon in chronic hepatitis B therapy.

Alcoholic Liver Disease

Liver diseases induced by excessive alcohol consumption are an important cause of morbidity and mortality worldwide. Alcoholic liver diseases can manifest themselves as one of the following disorders: alcoholic fatty liver, alcoholic hepatitis or alcohol-related cirrhosis. Alcoholic liver injury can develop into fibrosis or cirrhosis in up to 15% of alcoholics. On the other hand, alcoholic hepatitis and steatohepatitis are present in 35% of alcoholics.[7] Similarly, NAFLD encompasses a spectrum of disease from simple steatosis through non-alcoholic steatohepatitis (NASH) to fibrosis and ultimately cirrhosis and hepatocellular carcinoma.[8] Therefore, detection of an early stage of liver damage is the key to provide a positive outcome for therapeutic intervention.

Non-Alcoholic Fatty Liver Disease

The identification of the minority of patients with fibrosis amongst those with NAFLD is critically important for prognosis. Most causes of chronic liver disease share a similar clinical course with a prolonged asymptomatic early phase during which the risk of liver-related morbidity and mortality is minimal. Liver fibrosis accumulates silently during this early phase and may eventually progress to cirrhosis. Regardless of the aetiology of chronic liver disease, the risk of developing hepatocellular carcinoma, liver-related complications and liver-related death increases dramatically after the development of severe fibrosis and cirrhosis.

Decompensated cirrhosis occurs when clinically evident liver complications develop and this phase rapidly progresses towards death or liver transplantation. Risk factors for the progression to cirrhosis in patients with NAFLD include NASH, metabolic factors, genetic polymorphisms and older age.[9] NASH can progress to cirrhosis in up to 20% of patients.[10] The median survival for patients with decompensated cirrhosis is approximately two years.

A good representation of the mechanism of hepatic fibrogenesis is shown in **Figure 1**. Hepatic stellate cells are the key fibrogenic cells and their activation (conversion of quiescent, vitamin A-storing cells into proliferative, fibrogenic and contractile myofibroblasts which can synthesise and secrete large amounts of fibril-forming collagens) is the dominant event in fibrogenesis, as a response to liver injury.[3] Damaged hepatocytes release cytokines (transforming growth factor, tumour necrosis factor), epidermal growth factor and insulin-like growth factor responsible for the activation of Kupffer cells and the recruitment of activated T-cells. Activated Kupffer cells, T-cells and damaged hepatocytes also release the inflammatory cytokines (TNF-interferon, IL-6) which are closely related to fibrogenesis and could be used as biomarkers for liver fibrosis.[3]

Liver Biopsy as the Standard Test for Fibrosis

Although highly invasive and limited by sampling error and inter-observer variability, liver biopsy is still considered the gold standard for the staging of liver fibrosis. At present, several non-invasive methods for the assessment of liver fibrosis based on panels of serum markers, or the measurement of liver stiffness by radiography, are widely used as surrogate measures. However, these methods are also not free of limitations and many components of serum panels do not directly reflect the underlying disease process. With regard to liver biopsy, it is recognised that histologic staging is based on flawed assumptions, namely inappropriateness in describing a continuous variable such as the amount of fibrosis with categorical values such as the fibrosis stages. In addition, histologic staging systems assume a linear increase in the severity of fibrosis between stages, although it is widely recognised that stage 4 does not necessarily represent twice as much fibrosis as stage 2.[12] Algorithm scores, as continuous variables, may be better suited to describing fibrosis.

Alternatives to Biopsy

Noninvasive alternatives to biopsy include radiologic and serum tests. A number of different types of ultrasound-based

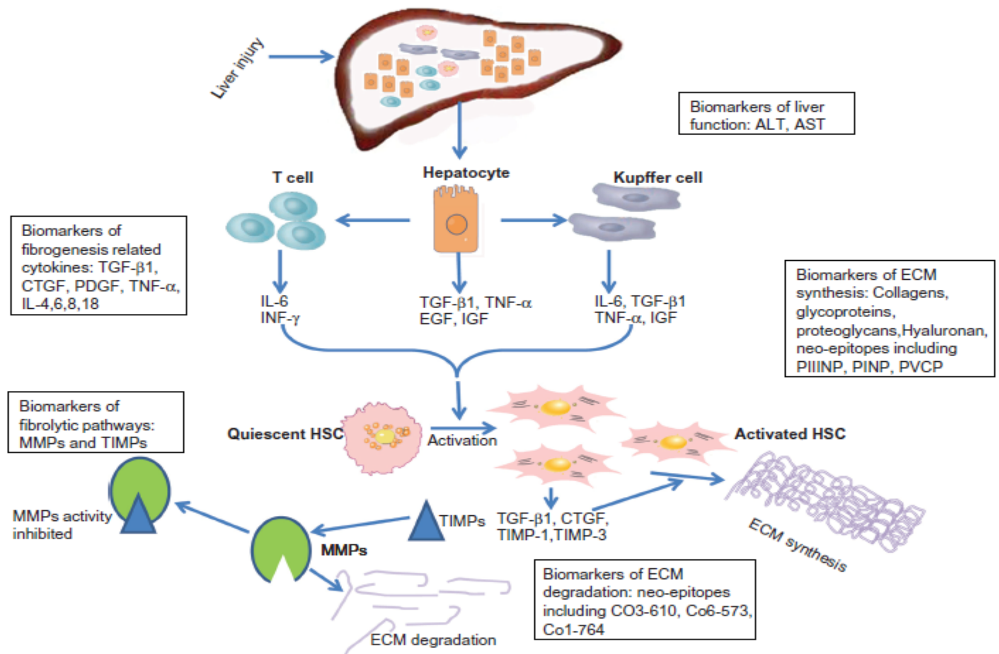

Figure 1. Mechanisms of hepatic fibrogenesis and possible molecular serum biomarkers. Some molecular serum biomarkers may reflect the pathogenesis of liver fibrosis: neo-epitopes are related to basement membrane degradation; pro-collagen is related to extracellular matrix (ECM) synthesis; matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) are related to ECM fibrolytic processes; ALT and AST are related to liver function and injury; other serum markers are fibrogenesis-related cytokines. Reproduced from Liu et al. Biomark Insights 2012;7:105-17 (ref. 11).

TGF-β1, transforming growth factor β1; CTGF, connective tissue growth factor; PDGF, platelet-derived growth factor; TNF-α, tumour necrosis factor α; IL, interleukin; INF-γ, interferon-γ; EGF, epidermal growth factor; IGF, insulin-like growth factor; PIIINP, procollagen III N-terminal peptide; PINP, procollagen I N-terminal peptide; PVCP, procollagen V C-terminal peptide; HSC, hepatic stellate cells; CO3, CO6, CO1, collagen fragments.

elastography methods (ultrasound transient elastography, acoustic radiation force impulse imaging and magnetic resonance elastography) have been developed to assess liver fibrosis and diagnose cirrhosis. Elastography measures tissue stiffness. Assessing liver fibrosis with ultrasound-based elastography measures only a small component of tissue stiffness.[13] Many other factors such as inflammation from acute hepatitis, cholestasis from biliary tract obstruction, blood congestion due to hepatic outflow obstruction, portal hypertension and food intake can affect liver tissue stiffness and have been shown to affect the accuracy of assessing liver fibrosis with elastography.[13]

Liver fibrosis markers are typically divided into indirect and direct markers of fibrogenesis and fibrinolysis. Indirect markers of hepatic fibrosis include biochemical tests that reflect alterations in hepatic function such as bilirubin, AST, ALT, GGT and platelet count. Direct markers of fibrosis, on the other hand, can be divided into markers associated with matrix deposition, degradation, cytokines and chemokines associated with the molecular pathogenesis of fibrogenesis and fibrinolysis. Examples include hyaluronic acid, alpha-2-macroglobulin, procollagen and laminin.

Several algorithms comprising panels of tests in combination have been validated in patients who have been staged for fibrosis using a liver biopsy (**Table**). Some diagnostic panels use only conventional direct markers, whilst most combine both direct and indirect markers, and others use only indirect markers.

Several systematic reviews and meta-analyses have sought to compare serum tests for staging fibrosis by describing the Area Under the Receiver Operating Characteristic curve (AUROC), which is a summary metric of the overall discriminatory power of a test comparing specificity and sensitivity irrespective of disease prevalence in a population.[26] Tests are generally evaluated on their ability to discriminate significant fibrosis (Metavir score ≥F2), advanced fibrosis (Metavir score ≥F3) or cirrhosis (Metavir score F4) . Other metrics of discriminatory power such as positive and negative predictive value are useful within specific populations, but are dependent on the population prevalence of liver fibrosis within a specific group. No panel has yet emerged as the standard of care, and the choice of panel is often dictated by local availability.

Table. Biomarkers of liver fibrosis.

Test	Markers	Reference
AST to platelet ratio index	AST, platelets	14
FibroTest, FibroSure	Age, sex, bilirubin, GGT, α2M, haptoglobin, apo-A1	15
Hepascore	Bilirubin, GGT, α2M, HA, age, sex	16
AST/ALT ratio	AST, ALT	14
FIB-4 index	Age, AST, ALT, platelets	17
NAFLD fibrosis score	Age, BMI, IFG/diabetes, AST/ALT ratio, platelets, albumin	18
PGA index	prothrombin time, GGT, apo-A1	19
FibroIndex	AST, platelets, γ-globulin	20
Forns index	Age, GGT, platelets, prothrombin, cholesterol	21
Fibrometer	Age, AST, platelets, α2M, HA, prothrombin index, urea	22
BARD score	AST/ALT ratio, BMI, diabetes	23
Proteomics and Glycomics	Various biomarker fragments	24
FibroSpect II	α2M, HA,TIMP-1	25
European Liver Fibrosis panel (ELF)	Age, HA, TIMP-1, PIIINP	12

Apo-A1, Apolipoprotein A-1; α2M, α2-macroglobulin; HA, hyaluronic acid; BMI, body mass index; TIMP-1, tissue inhibitor of metalloproteinase 1; PIIINP, procollagen III N-terminal peptide.

Future Developments

Proteomics is an emerging field that uses many types of proteomic platforms which are being used to identify novel biomarkers, offering the potential to further increase the accuracy and clinical utility of fibrosis biomarker models.[7] Application of genomic medicine to the field of fibrosis prediction has highlighted the variation in genetic susceptibility and fibrosis rates between individuals. Further refinement of genetic risk scores and their incorporation with more routinely available fibrosis biomarkers offer the potential to individualise fibrosis risk prediction, thereby introducing powerful prognostic tools for liver morbidity and mortality. However, few standardised procedures exist and attention has been drawn to the reliability of the results published in the literature.[27]

Proteomics has the potential to increase knowledge of the biology of liver fibrogenesis by assessing patterns of proteins or glycoproteins by mass spectroscopy in serum samples. Surface-enhanced laser desorption/ionisation (SELDI) is a variation of matrix-assisted laser desorption/ionisation (MALDI) and has been widely used in discovery studies to identify new protein molecular markers. SELDI can be described as a combination of solid-phase chromatography and TOF-MS. In limited studies published to date, these have been demonstrated to be highly predictive (AUROC >0.85) of fibrosis in hepatitis C, hepatitis B and NAFLD.[28]

Recent advances in genomics and the advent of new efficient tools for large-scale analysis of gene expression have provided new insights into the knowledge and understanding of gene networks and regulatory pathways in various disease processes.[29] These methods include microarrays which can be used to analyse the expression of thousands of genes at a time. Currently, however, the cost and technology involved prohibit routine use of these methods. What is also lacking is a rigorous, independent and widespread evaluation of the utility of the already proposed biomarkers and new suggested biomarkers in the diagnosis and follow-up of chronic liver diseases before they can be recommended for use in routine clinical practice.

Eslam *et al*. suggest that the incorporation of an invariant genetic marker of liver fibrosis risk to algorithms for fibrosis prediction could be useful.[30] They reported that a single-nucleotide polymorphism (rs12979860) in the intronic region of the interferon lambda 4 (IFNL4) gene modulates liver inflammation and fibrosis in a manner independent of the aetiology of the fibrosis. Thus, it could be hypothesised that this polymorphism, for which a test is widely used and commonly available, might have a role in algorithms that predict fibrosis. The FibroGENE-DT which uses this polymorphism yielded AUROCs of 0.87, 0.85 and 0.804 for the prediction of fast fibrosis progression, cirrhosis and significant fibrosis risk, respectively, with comparable results in the validation cohort. The model performed well in NAFLD and chronic hepatitis B with AUROCs of 0.791 and 0.726, respectively. The negative predictive value to exclude cirrhosis was >0.96 in all three liver diseases.

Yet another development, clinical glycomics, is a DNA sequencer/fragment analyser-based N-glycan profiling tool where desialylated serum protein N-glycan profile is proving to be an excellent biomarker for the detection of liver cirrhosis (**Figure 2**). Further development looking at the ratios of just two peaks in the profile (renamed GlycoCirrho Test) shows promise.[24]

Marker Panels as Predictors of Clinical Outcome

The development of simple serum liver panel models that are able to stratify chronic hepatitis C patients into a hierarchy of risk levels of adverse clinical outcomes, the liver outcome score (LOS), is of considerable clinical significance. The LOS has a high degree of accuracy in predicting five-year liver-related death, liver decompensation and hepatocellular carcinoma when compared to Hepascore, AST to platelet ratio index (APRI) and fibrosis-4 (FIB-4).[31]

A long-term follow up of patients was obtained from a population-based data linkage system that linked health-related datasets including the state cancer register, hospital morbidity database and the mortality records. The predictive ability of the LOS was better than the currently used fibrosis models. The use of the LOS panels will potentially improve clinical care by allowing the optimum use of expensive DAA agents before the onset of significant clinical complications. In addition, these models would potentially be valuable in determining the start of ultrasound screening for hepatocellular carcinoma and for assessing the presence of complications of portal hypertension. Future studies are required to validate these models in addition to the presently accepted clinical criteria used in chronic hepatitis C patients.

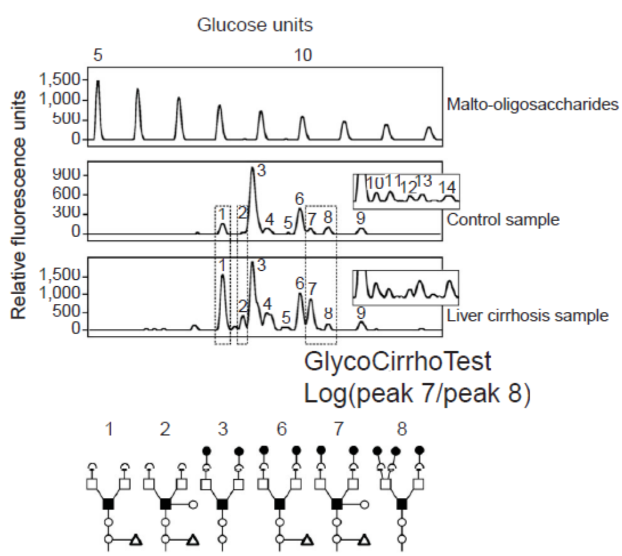

Figure 2. Examples of total serum protein N-glycan profiles. Electropherograms: top, malto-oligosaccharide reference; middle, typical electropherogram of desialylated N-glycans derived from proteins in control serum sample (nine peaks are clearly visible in the full detection range, with five more in the x10 blow-up of the latter part of the electropherogram); bottom, representative electropherogram obtained from a cirrhosis case. Structures of N-glycans of relevance to this study are shown below the panels.

Conclusion

Using multiple serum panels or combining serum panels with radiographic imaging may improve the ability to correctly assess the degree of a patient's fibrosis compared to liver biopsy. In addition, it may be possible to improve the diagnostic performance of these panels if they are used in stepwise combination.[32] Noninvasive markers of fibrosis are being incorporated into the routine clinical care of patients with liver disease and this field continues to evolve.

With the availability of accurate noninvasive tests, the ability to screen large cohorts for significant liver disease is now becoming possible, allowing the assessment of the true burden of liver disease in the general population for the first time. Moreover, as novel anti-fibrosis therapies enter clinical trials, robust noninvasive markers are crucial to allow effective trial design and obviate the need for multiple invasive liver biopsies to assess efficacy.[12]

Competing Interests: None declared.

References

1. Alazawi W, Cunningham M, Dearden J, Foster GR. Systematic review: outcome of compensated cirrhosis due to chronic hepatitis C infection. Aliment Pharmacol Ther 2010;32:344-55.
2. Hepatitis Australia. https://www.hepatitisaustralia.com/ (Accessed 4 May 2020).
3. Liu T, Wang X, Karsdal MA, Leeming DJ, Genovese F. Molecular serum markers of liver fibrosis. Biomark Insights 2012;7:105-17.

4. Gastroenterological Society of Australia. Economic cost and health burden of liver disease in Australia. https://www.gesa.org.au/index.cfm//resources/economic-cost-and-health-burden-of-liver-disease-in-australia/ (Accessed 30 April 2020).
5. Australian recommendations for the management of hepatitis C virus infection: a consensus statement (September 2018); Gastroenterological Society of Australia. https://www.asid.net.au/documents/item/1208 (Accessed 4 May 2020).
6. Direct acting antiviral medicines for the treatment of chronic hepatitis C. https://www.pbs.gov.au/industry/listing/participants/public-release-docs/2018-09/hepatitis-c-review-prd-2018-19.pdf (Accessed 4 May 2020).
7. Chrostek L, Przekop D, Gruszewska E, Gudowska-Sawczuk M, Cylwik B. Noninvasive indirect markers of liver fibrosis in alcoholics. Biomed Res Int 2019;3646975.
8. Guha IN, Parkes J, Roderick P, Chattopadhyay D, Cross R, Harris S, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis panel and exploring simple markers. Hepatology 2008;47:455-60.
9. Li B, Zhang C, Zhan YT. Nonalcoholic fatty liver disease cirrhosis: a review of its epidemiology, risk factors, clinical presentation, diagnosis, management, and prognosis. Can J Gastroenterol and Hepatol 2018:2784537.
10. Mehta R, Younossi ZM. Natural history of nonalcoholic fatty liver disease. Clinical Liver Disease 2012;1:112-3.
11. Liu T, Wang X, Karsdal MA, Leeming DJ, Genovese F. Molecular serum markers of liver fibrosis. Biomark Insights 2012;7:105-17.
12. Rosenberg WMC, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, et al. Serum markers detect the presence of liver fibrosis: cohort study. Gastroenterology 2004;127:1704-13.
13. Chin JL, Pavlides M, Moolla A, Ryan JD. Non-invasive markers of liver fibrosis: adjuncts or alternatives to liver biopsy? Front Pharmacol 2016;7,159.
14. Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998;93:44-8.
15. Salkic NN, Jovanovic P, Hauser G, Brcic M. FibroTest/Fibrosure for significant liver fibrosis and cirrhosis in chronic hepatitis B: a meta-analysis. Am J Gastroenterol 2014;109:796-809.
16. Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, et al. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. Clin Chem 2005;51:1867-73.
17. Vallet-Pichard A, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection: comparison with liver biopsy and fibrotest. Hepatology 2007;46:32-6.
18. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846-54.
19. Poynard T, Aubert A, Bedossa P, Abella A, Naveau S, Paraf F, et al. A simple biological index for detection of alcoholic liver disease in drinkers. Gastroenterology 1991;100:1397-402.
20. Koda M, Matunaga Y, Kawakami M, Kishimoto Y, Suou T, Murawaki Y. FibroIndex, a practical index for predicting significant fibrosis in patients with chronic hepatitis C. Hepatology 2007;45:297-306.
21. Forns X, Ampurdanès S, Llovet JM, Aponte J, Quinto L, Martinez-Bauer E, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 2002;36:986-92.
22. Calès P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, et al. A novel panel of blood markers to assess the degree of liver fibrosis. Hepatology 2005;42:1373-81.
23. Harrison SA, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441-7.
24. Callewaert N, Van Vlierberghe H, Van Hecke A, Laroy W, Delanghe J, Contreras R. Noninvasive diagnosis of liver cirrhosis using DNA sequencer-based total serum protein glycomics. Nat Med 2004;10:429-34.
25. Patel K, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, et al. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. J Hepatol 2004;41:935-42.
26. Non-invasive Testing for Liver Fibrosis; Johns Hopkins HealthCare LLC Medical Policy. https://www.hopkinsmedicine.org/johns_hopkins_healthcare/downloads/Policies/cms_03_13_noninvasive_testing_for_liver_fibrosis_2018.pdf (Accessed 30 April 2020).
27. Mas VR, Fisher RA, Archer KJ, Maluf DG. Proteomics and liver fibrosis: identifying markers of fibrogenesis. Expert Rev Proteomics 2009;6:421-31.
28. Karatayli SC, Alagöz SG, Mizrak D, Sayki M, Ozkan M, Savaş B, et al. Potential proteomic biomarkers in assessing liver fibrosis using SELDI-TOF MS. Turk J Gastroenterol 2012;23:46-53.
29. van't Veer LJ, Bernards R. Enabling personalized cancer medicine through analysis of gene-expression patterns.

Nature 2008;452:564-70.
30. Eslam M, Hashem AM, Romero-Gomez M, Berg T, Dore GJ, Mangia A, et al. FibroGENE: A gene-based model for staging liver fibrosis. J Hepatol 2016;64:390-8.
31. Huang Y, Adams LA, MacQuillan G, Speers D, Joseph J, Bulsara MK, et al. Serum models accurately predict liver-related clinical outcomes in chronic hepatitis C. J Gastroenterol Hepatol 2016;31:1736-41.
32. Castéra L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 2005;128:343-50.